(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 020 029**
**A1**

# (12) EUROPEAN PATENT APPLICATION

(21) Application number: **80301443.0**

(22) Date of filing: **01.05.80**

(51) Int. Cl.³: **C 07 J 71/00**, C 07 J 43/00, A 61 K 31/58, A 61 K 31/70, A 61 K 35/78

(30) Priority: **02.05.79 AU 8621/79**

(43) Date of publication of application: **10.12.80 Bulletin 80/25**

(84) Designated Contracting States: **AT BE CH DE FR GB IT LI LU NL SE**

(71) Applicant: **ARUBA (QLD.) PTY. LTD., c/o O.W. Gerke & Co. Quantas House Queen Street, Brisbane 4000 Queensland (AU)**

(72) Inventor: **Cham, Bill Elliott, 772 Upper Brookfield Road, Upper Brookfield 4069, Queensland (AU)**
Inventor: **Gerns, Edwin Harold John, 76 Holme Avenue, Boondall 4034, Queensland (AU)**
Inventor: **Gerns, Henry Harol, 4, Buhot Street, Geebung 4034, Queensland (AU)**

(74) Representative: **Silverman, Warren et al, HASELTINE LAKE & CO. Hazlitt House 28 Southampton Buildings Chancery Lane, London WC2A 1AT (GB)**

## (54) Steroid alkaloids, process for their extraction and their pharmaceutical compositions.

(57) The compounds and compositions of the present invention provide for another efficient chemotherapeutic agent for the treatment of cancer. The compounds of the present invention may be derived from extracts of the Solanum plant family. Extracts of one such plant (Solanum sodomeum) known in Australia as apple of Sodom, and incorrectly as devil's apple will be described by way of an example to illustrate the present application. This plant bears fruit which is similar in shape to, but smaller than an apple. The fruit of the plant is generally considered to be poisonous.

EP 0 020 029 A1

0020029

TITLE MODIFIED
see front page

# MEDICATION

The present invention relates to steroid alkaloids, pharmaceutical compositions containing same and therapeutic methods employing steroid alkaloids.

The compounds and compositions of the invention have been found to be useful in chemotherapeutic treatment of cancers, both neoplastic and granulomatous, of skin inflammations of mycotic infections such as tinea, of non-malignant dermatitis such as psoriasis, of haemorrhoids and of acne, and for other cosmetic uses.

The chemotherapy of cancers (with drugs) goes back into antiquity. Early Egyptian papyruses describe the application of various mixtures of drugs to ulcerating skin tumours. However, the emergence of chemotherapy as an effective modality of cancer treatment in modern medicine is a relatively recent development. Emphasis on chemotherapy as a primary modality for therapy has also come about with the realisation that cancer is often a systemic disease where local forms of treatment are often inadequate.

Large numbers of compounds have been studied in the search for new drugs with improved therapeutic efficacy. As a result of these efforts, several different classes of chemotherapeutic agents have been identified. The availability of a variety of drugs with different mechanisms of action and with differing host toxicities has provided a new dimension for the role of chemotherapy in the treatment of cancer.

The alkylating agents which include nitrogen mustard, cyclophosphamide, chlorambucil, molphalan, and busulfan, are among the oldest and most established drugs used for the treatment of cancer.

Antimetabolic agents have been defined and comprise another class of anti-cancer drugs. Methotrexate, fluoro-uracil, mercaptopurine, thioguanine, and cytosine arabinoside are included in this class of potent anti-tumour drugs.

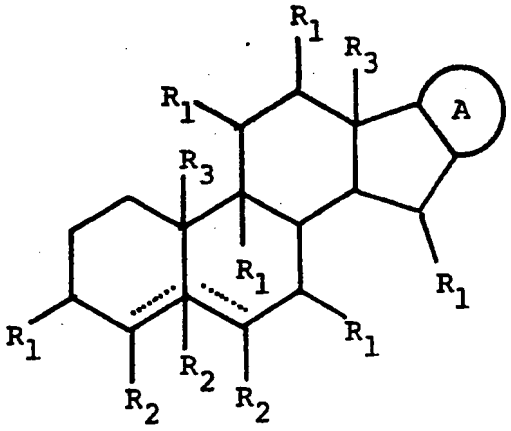
Yet another class of agents found to have significant anti-tumour activity are the antibiotics, products of

living organisms that have profound anti-tumour properties. Among these are drugs such as actinomycin D, mitramycin, daunoribicin, mitomycin C, and adriamycin.

Folklore has provided a different type of chemotherapeutic agent, the vinca alkaloids. Vincristine and vinblastine (U.S. patent 3,225,030 in 1965 by Eli Lilly), extracts of the periwinkle plant (Vinca Rosea), have become firmly established in the treatment of acute leukemia and other types of cancer.

The compounds and compositions of the present invention provide for another efficient chemotherapeutic agent for the treatment of cancer. The compounds of the present invention may be derived from extracts of the Solanum plant family. Extracts of one such plant (Solanum sodomeum) known in Australia as apple of Sodom, and incorrectly as devil's apple will be described by way of an example to illustrate the present application. This plant bears fruit which is similar in shape to, but smaller than an apple. The fruit of the plant is generally considered to be poisonous.

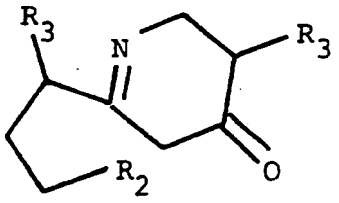
The present invention therefore provides compounds of the formula



(I)

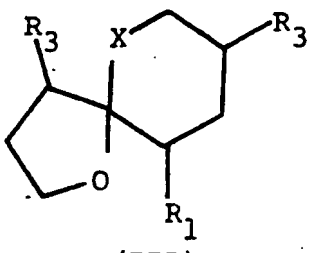
wherein one of the dotted lines represents a double bond, or both represent single bonds;

A represents



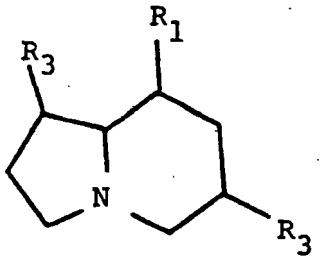
(II)

,



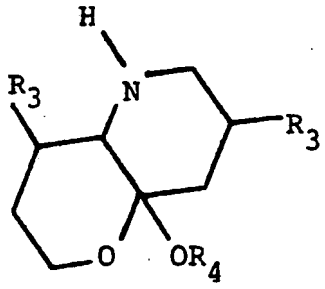
(III)

,



(IV)

or



(V)

;

$R_1$ represents hydrogen, amino, oxo or $OR_4$;

$R_2$ represents hydrogen, amino or $OR_4$ provided that $R_2$ represents hydrogen when adjacent a double bond and no more than one $R_2$ is other than hydrogen;

$R_3$ represents hydrogen, $(C_1-C_6)$ alkyl or $R_4O(C_1-C_6)$ alkylene;

$R_4$ represents hydrogen, a tetrose, pentose or hexose, or two, three, four or more linked units, wherein each unit is independently selected from tetroses, pentoses and hexoses; and

X represents O or NH;

and the pharmaceutically acceptable salts of such compounds; with the proviso that no more than two of $R_1$ and $R_2$ are other than hydrogen.

It will be understood that in formulae (I), (II), (III), (IV) and (V) above stereochemistry is not represented. The terms "tetrose", "pentose" and "hexose" include the deoxy derivitives thereof, e.g. rhamnose.

The invention also provides pharmaceutical compositions comprising as active ingredient, an effective amount of at least one compound of formula (I) above, or pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier.

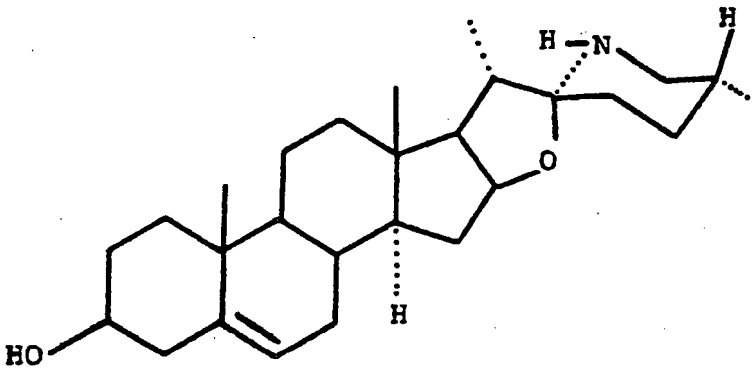
The method of the present invention comprises administering to a subject having the disorder to be treated an effective amount of at least one compound of formula I above or the composition as outlined above.

The compounds of formula (I) are steroid alkaloids and their glycosides and as indicated above the glycosides

may be monoglycosides, diglycosides, triglycosides, tetraglycosides or polyglycosides.

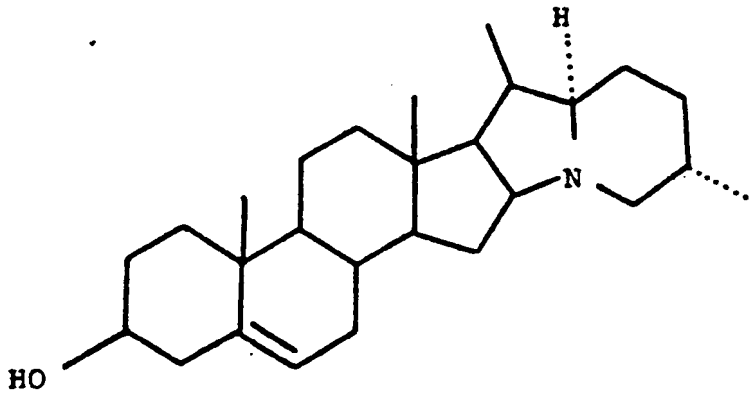
Representative of the aglycones of the compounds of the present invention are solasodine (VI), solanidine (VII), diosgenin, tomatidine, solangustidine, leptinidine, solacongestidine, solafloridine, demissidine, soladulcidine, tomatidenol, paniculidine, jurjubidine, tigogenin, yamogenin and neotigogenin.

The aglycones isolated from Solanum sodomeum are




(VI)

solasodine, and



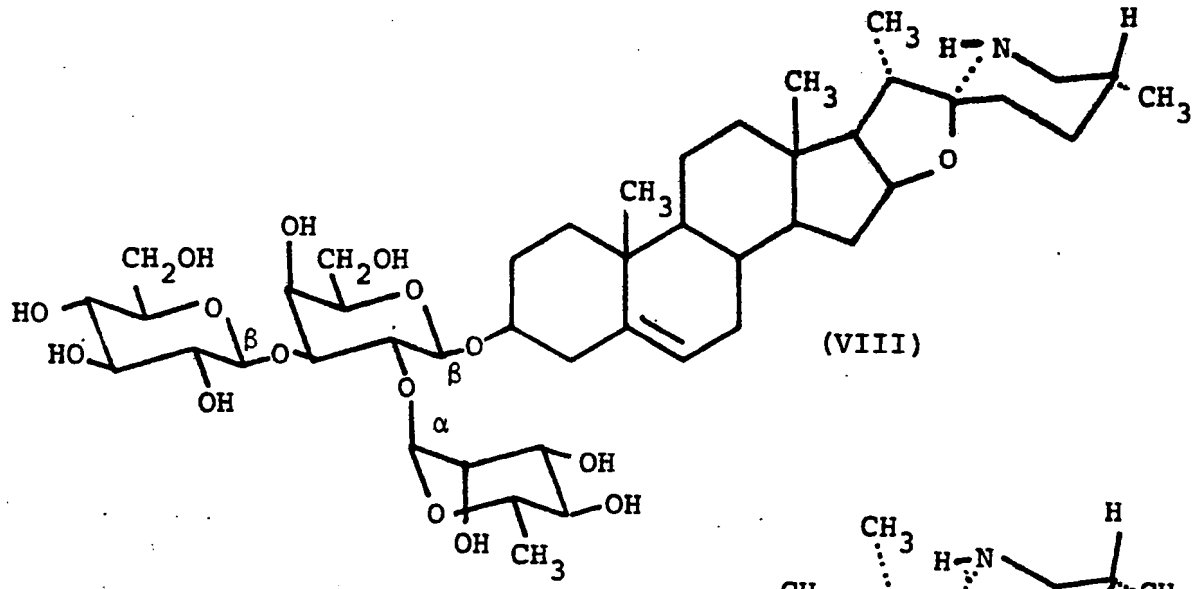

(VII)

solanidine.

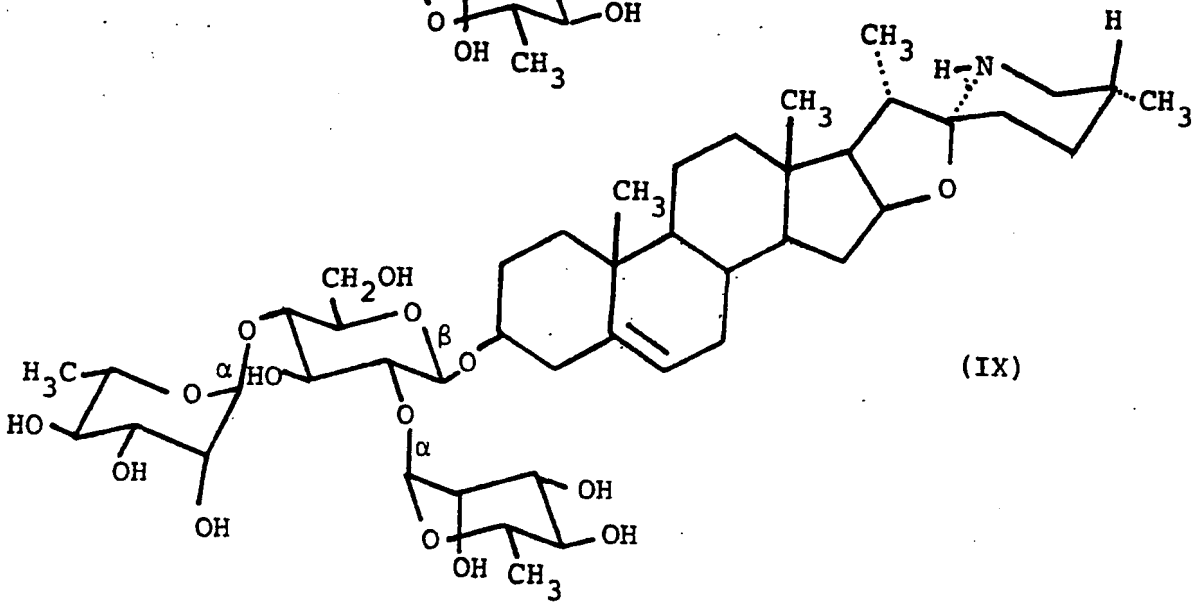
When the compounds of formula I represent glycosides, as indicated above, they may be mono-, di-, tri-, tetra-, or polyglycosides and may be derived from tetroses such as erythrose and threose; pentoses such as ribose, arabinose, xylose and lyxose; and hexoses such as allose, altrose, glucose, mannose, gulose, idose, galactose, talose, fructose, sorbose, tagatose and psicose.

The sugar units in the di-, tri-, tetra-, and polyglycosides are linked to each other in known manner. The hexoses may, for example, have 1,5 or 1,4 linkages.

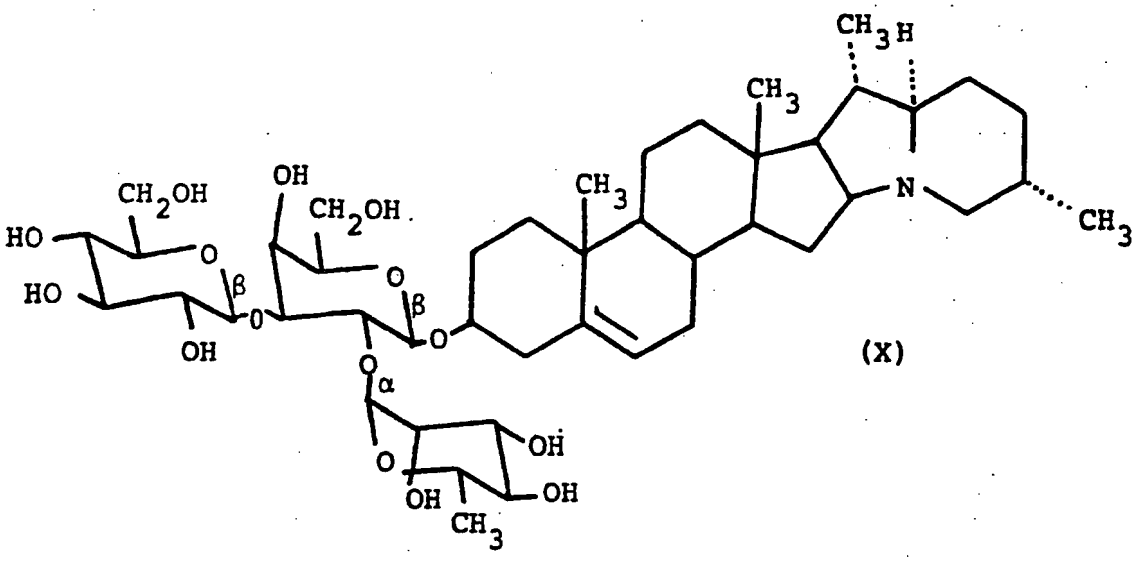
Three glycoalkaloids can be isolated from Solanum sodomeum, solasonine (VIII), solamargine (IX) and solanine (X)



(VIII)



(IX)



(X)

The glycoalkaloids can be extracted from plants of the Solanum species by grinding any parts of the plant and subjecting the ground plant matter to the action of dilute acid, and making the acid extracts alkaline to precipitate the glycoalkaloids.

The following examples illustrate extraction of a mixture of the glycoalkaloids (VIII), (IX) and (X) from Solanum sodomeum.

Example 1

Plant material from Solanum sodomeum is coarsely ground and then mixed with ten volumes (w/v) of 2% acetic (or formic) acid and shaken for two to four hours. This is then coarsely filtered (or centrifuged) and the residue is reshaken with ten volumes of 2% acetic (or formic) acid for another two to four hours at room temperature. The second extract is filtered and both solutions are added together and centrifuged to remove the last traces of residue. The solutions are made alkaline with ammonia, and heated to 80°C which causes a precipitate to form. This precipitate is dissolved in boiling alcohol and filtered while boiling. The alcohol is then evaporated to dryness. This yields a fine powder.

Example 2

Plant material from Solanum sodomeum is coarsely

ground with two volumes (w/v) or 3% aqueous acetic acid in a Waring blender. The mixture is diluted with another two volumes of 3% aqueous acetic acid and is then shaken for eighteen to twenty hours at room temperature, then filtered through muslin. Two litre aliquots of the filtrate is heated to 50°C with continual stirring and then concentrated ammonia solution is added until the pH = 9-10 (approx. 50 ml/ litre). The solution is maintained at 50°C for a further five minutes, allowed to cool and then centrifuged. The supernatant is discarded and the precipitate is dissolved in 1 litre 3% aqueous acetic acid. The solution is centrifuged and the supernatant heated to 50°C with continual stirring. The glycoalkaloids are reprecipitated on addition of concentrated ammonia solution until the pH = 9-10. The solution is maintained at 50°C for a further five minutes as before, allowed to boil and then centrifuged. The glycoalkaloids precipitate is dried overnight at 50°C and then extracted with 100 ml boiling ethanol. The ethanolic solution is centrifuged and the supernatant dried at 50°C. This yields a fine powder.

The extract as prepared in Examples 1 and 2 will hereinafter be referred to as BEC 001.

Other plant species of the Solanum family may be extracted in a similar manner to that described in Examples 1 and 2 to produce a powder which contains various alkaloids. The composition of the alkaloids is dependent on the species of plant material which is used for extraction.

The various glycoalkaloids can be separated by HPLC methods which are known.

The following example illustrates separation of the glycoalkaloids (VIII), (IX) and (X) from the BEC 001.

Example 3

The powder extract from Solanum sodomeum (BEC 001) was dissolved in an eluent containing acetonitrile/$B_5$ (Pic Reagent)/triethanolamine (83:17:0.1) adjusted to pH 2.7 - 3.0 with concentrated phosphoric acid, at a concentration of 0.1% (by weight).

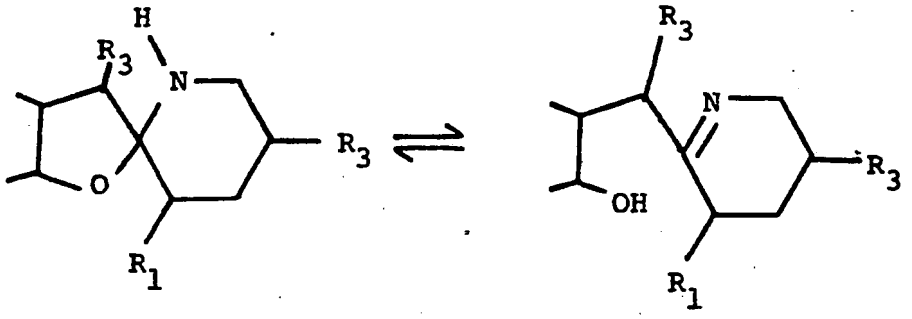
50µl samples were applied to an injector (Model U6K universal injector*) and was chromatographed at ambient temperature on a 30cm x 3.9 mm "carbohydrate analysis"

or an "-$NH_2$" prepacked column*, average particle size 10μm at a flow rate of 2 ml per minute (Model 6000A solvent delivery system*). A model 450 variable wavelength detector*, with sensitivity set at 0.01 Aufs, was used and peak areas at 205 nm were recorded with a 10mV omniscribe recorder. (*Equipment obtained from Waters Associates Inc.).

Suitable pharmaceutically acceptable salts of the compounds of formula I are, e.g. the hydrochlorides and hydrogen sulfates.

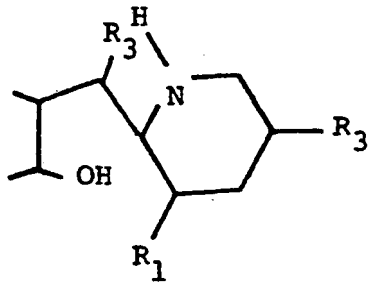
Compounds of formula (I) may be transformed from one to another in various manners. For example, when $R_4$ represents a sugar moiety, the compound may be hydrolysed to the corresponding aglycone which may be then reacted with a different sugar in the presence of a dehydrating agent. Where a compound of formula (I) has a double bond at 4 or 5, it may be hydrogenated to the corresponding saturated compound or $HOR_4$ can be added across the double bond.

In compounds of formula (1) wherein A represents a group of formula III in which X is NH, an equilibrium exists with compounds in which a is in one form of formula II (designated IIA).



(III) (IIA)

Such compounds can be reduced to corresponding compounds of formula IIB.



(IIB)

Compounds in which A is of formula IIB can be transformed to those in which A is of formula (IV) by oxidation followed by reduction.

Compounds of formula (I) wherein A represents a group of formula III in which X is NH can be converted to those in which X is O by deamination of the N-nitroso derivatives, e.g. with 30% aqueous acetic acid in ethanol.

The chemistry of the steroid alkaloids and their glycosides which are derived from the Solanum group is discussed in detail by Prelog and Jeger, The Alkaloids, Ed. Manske Vol. III, 247 (1952); idem, ibid, Vol. VII, 243 (1957); and Schreiber, ibid, Vol X, 1 (1968); the disclosure of which is incorporated herein by reference.

The steroid alkaloids and their glycosides of formula I hereinbefore may be used in the chemotherapeutic treatment of cancers, both neoplastic and granulomatous, of skin inflammation, of mycotic infections such as tinea and ringworms, of viral infections such as warts, of haemarrhoids, of bacterial infections such as acne, and of non malignant dermatitis such as psoriasis, and for other cosmetic uses. They may also be used as adjuncts to topical antifungal and antibacterial preparations.

The steroid alkaloids and their glycosides of formula I hereinbefore may be used as medicaments in the form of pharmaceutical preparations which contain them without, or in association with a compatible pharmaceutical carrier material, or for synergistic use with other chemotherapeutic agents. The carrier material may be an organic or inorganic inert carrier material suitable for internal (e.g. oral), external (e.g. dermal), or parenteral administration. Examples of such carrier materials include dimethylsulfoxide, water, lactose, starch, magnesium stearate, talc, gum arabic, gelatin, polyalkylene glycols and petroleum jelly. The pharmaceutical preparations can be made up in a solid form, e.g., tablets, dragees, suppositories or capsules, or in liquid form, e.g., as solutions, emulsions, suspensions or aerosols. The pharmaceutical preparations may be subjected to customary pharmaceutical operations, such as sterilisation and may contain adjuvants such as preservatives,

stabilisers, wetting agents, buffers and salts for varying osmotic pressure.

Alternatively the steroid alkaloids may be formulated in suitable pharmaceutical vehicles for topical application e.g., as lotions, ointments or creams by incorporating them in conventional lotion, ointment or cream bases, such as zinc oxide, alkyl polyether alcohols, cetyl alcohol, stearyl alcohol or polyethylene glycol 1000 monoacetyl ether (cetomacrogol). They may also be formulated as solutions in dimethylsulfoxide. Other solid forms include powders wherein the steroid alkaloids are incorporated in conventional powder bases such as starch or talc or jellies in which the base is, e.g. glycerol or tragacanth.

The following example illustrates toxicity tests on BEC 001 as prepared in Examples 1 and 2.

Example 4

Single Dose

The toxicity of a single ip dose of BEC 001 in mice is illustrated in fgiure 1. It can be seen that the $LD_{50}$=30 mg/kg (mg BEC 001 per kg mouse body weight). Toxicity studies of glycoalkaloids extracted from Solanum tuburosum which contains mainly solanine were conducted by Patil et al., {Food and Cosmetics Toxicology 10,395 (1972)} who found an $LD_{50}$ in mice (ip administation) of 32.3 mg/kg. Nishie et al {Toxicology and Applied Pharmacology 19,81 (1971)} reported an ip $LD_{50}$ of 42±1.8 mg/kg in mice, whereas Gull et al {Hortscience, 5,316 (1970)} found an ip $LD_{50}$ of 75 mg/kg when the pure alkaloid was used. The values calculated from experimental data on BEC 001 agree closely with those of Patil et al and Nishie et al. The postmortem examinations following administration of BEC 001, like those reported by Gull et al, revealed no well-defined symptoms directly attributable to the toxic effect of the glycoalkaloids. Patil et al found that administration of an ip dose in mice of over 50 mg solanine/kg was lethal within 1 - 3 hr, but a dose of 10 mg/kg caused no deaths; this is in agreement with results found. It was found that the $LD_{50}$ for gastric intubation in mice was 550 mg BEC 001 per kilogram, this is in close agreement with the value reported by Gull et al of 590 mg solanine per kilogram.

The $LD_{50}$ of BEC 001 for rates was found to be 41 mg/kg.

Multiple Doses

The $LD_{60}$ for mice by 14 ip administrations over 14 days (one injection per day) was 10 mg/kg.

The $LD_{50}$ for rats by 8 ip administrations over 8 days (one injection per day) was 20 mg/kg.

Local toxicity in mice

Intact and abraded skin.

The hair on the back of mice was clipped with electric clippers. The area of the hair removed was about 2.5 cm x 4.0 cm. Care was taken to avoid injury in a batch of 24 mice (batch A). Superficial skin injury using electric clippers was deliberately done on another batch of 24 mice (batch B). The average weight of mice was initially 40 g per mouse (36-44g). Batch A was divided into $A_1$ and $A_2$ of 12 mice each. Similarly batch B was divided into $B_1$ and $B_2$. Ten microlitres of dimethylsulfoxide (DMSO) was applied daily to the backs of mice in batches $A_1$ and $B_1$. Ten microlitres of DMSO containing 5% BEC 001 was applied to batches $A_2$ and $B_2$ daily. This study was continued for 16 weeks. At the end of the 16 weeks all mice grew normal hair and the skin appeared normal. A group of 5 mice of each batch was then sacrificed. Post mortem investigation revealed that there was no apparent gross abnormality of thoracic or abdominal contents.

Daily doses of approximately 12.5 mg BEC 001 per kg body weight applied topically to intact or abraded skin of mice for 16 weeks did not produce any obvious adverse effects.

Side effects

Patil et al reported that solanine appeared to be a weak-to-moderate inhibitor of both specific and non-specific cholinesterase. Following small multiple doses of solanine, a quick inhibition followed by rapid recovery of serum cholinesterase was noted in the dog. Red-cell cholinesterase was not inhibited. It was speculated that while small doses of solanine may cause discomfort upon ingestion, repeated doses will have little noticeable effect

resulting from acetylcholinesterase inhibition. Further studies by Patril et al revealed that atropine appeared to be antagonistic to the toxicity of solanine.

In human experiments Ruhl {Archiv der Pharmazie 284,67 (1961)} indicated that an oral dose of 200 mg solanine caused hyperesthesia, drowsiness, itchiness in the neck region, and dyspnea: higher doses caused vomiting and diarrhoea. It has been found that an oral dose of 250 mg BEC 001 caused no such effects in a human subject.

The following example illustrates the effect of BEC 001 on sarcoma 180 activity in mice.

Example 5

A suspension of sarcoma 180 cells was taken from the peritoneal fluid of a host mouse and a known aliquot was injected ip into the experimental mice to produce sarcoma 180 activity in the ascitic fluid. BEC 001 was made up as a 5% stock solution in dimethylsulfoxide (DMSO). The necessary control experiments was also conducted. Treatment of the mice was done by ip injections with appropriate quantities of BEC 001.

Figure 2 illustrates the inhibition of the activity of sarcoma 180 by BEC 001 (expressed as % survival of mice due to BEC 001). Mice inoculated with sarcoma 180 cells generally died in two to three weeks after inoculation. The criterion of survival was arbitrarily taken as eight weeks. This is 4 times normal time to death after inoculation of sarcoma 180 control mice untreated with BEC 001. It can be seen in figure 2 that a single administration of BEC 001 has an $ED_{50}$ of 10 mg/kg (i.e. activity of sarcoma 180 was inhibited in 50% of the animals of this dose).

Inhibition of the lethal effect of sarcoma 180 activity in mice was dependent on the number of doses of BEC 001. Figure 3 depicts the effect of the number of administrations of BEC 001 at a concentration of 8 mg/kg on the inhibition of the mice from this cancer type. Two doses achieved 42% inhibition whereas with three and four doses almost complete inhibition was obtained.

Figure 4 illustrates the results obtained in the test on the inhibition of sarcoma 180 activity in the

ascitic fluid of recipient mice using BEC 001. Five groups of twelve mice were used in the test. Curve Δ represents 1 injection of 8 mg/kg; Curve ● represents 2 injections of 8 mg/kg; Curve □ represents 3 injections of 8 mg/kg; Curve ▲ represents 4 injections of 8 mg/kg. The ip injections were done on consecutive days.

In summary; these preliminary studies indicate that BEC 001 is very effective in producing a highly significant inhibitory activity on the terminal cancer sarcoma 180. The efficacy and toxicity of BEC 001 depends on the route and type of application and is species dependent.

The following example illustrates the effect of BEC 001 on skin tumours.

Example 6

Preliminary studies of BEC 001 have shown exceedingly promising results in the treatment of skin cancers. Various preparations have been investigated ranging from crude plant extract (macerated fruit), BEC 001 in DMSO, BEC 001 in paraffin, BEC 001 in zinc ointment, BEC 001 in zinc cream, and BEC 001 in cetomacrogol. Studies done so far on a limited number of patients with skin tumours indicate that BEC 001 is effective to the types studied, viz, keratoses, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC).

Concentrations ranging from 0.1% to 50% BEC 001 have been studied on the skin in man. No apparent side effects were observed.

The preliminary studies indicate that the following formulation may be used to obtain satisfactory results.

| | | |
|---|---|---|
| BEC 001 | 4% | |
| DMSO | 5% | |
| Cetomacrogol | 91% | all in W/W |

One subject who originally had over 200 skin tumours has been using this preparation for over ten months and as far as can be determined has shown no ill effects.

One investigator has been applying 80 mg BEC 001, in the ointment form, twice daily for three months on himself and has encountered no apparent ill effects. An oral dose of 200 mg of BEC 001 did not affect the subject adversely. Biochemical (SNAC 20) and haematological

(coulter screen, platelet count, differential W.C.C.) tests revealed no significant changes due to BEC 001.

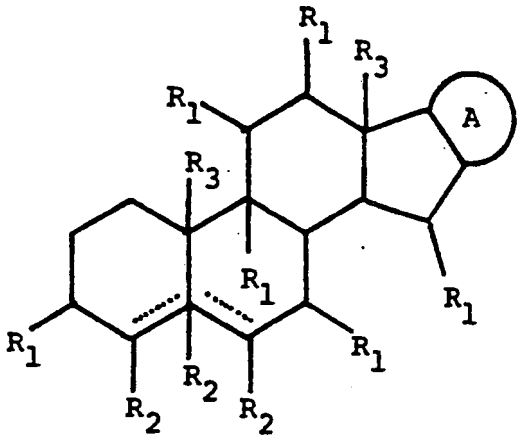
Two grams of the ointment are adequate to apply to 25 cm x 25 cm = 625 $cm^2$ area on the skin in man. This corresponds to 1.6 mg glycoalkaloid per kg body weight for a person weighing 50 kg. This refers to topical application. The absorption kinetics of this drug through the skin is not known at present.

Oral toxicity studies in mice have shown that the $LD_{50}$ is approximately 550 mg/kg.

Daily doses of approximately 12.5 mg BEC 001 per kg body weight applied topically to intact or abraded skin of mice for 16 weeks did not produce any obvious adverse effects.

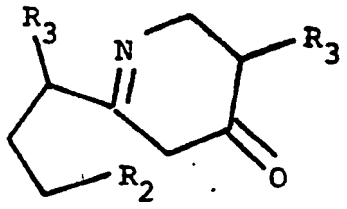
CLAIMS (all designated countries except Austria):-

1. Compounds of the formula

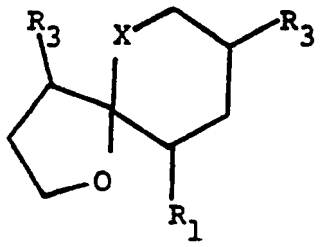


(I)

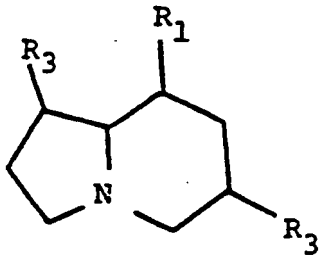
wherein one of the dotted lines represents a double bond, or both represent single bonds;
A represents



(II)

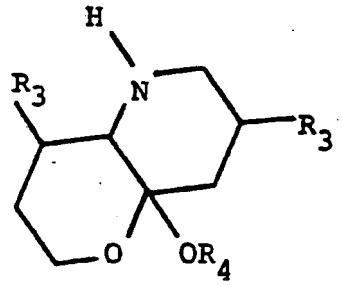


(III)



(IV)

or



(V)

;

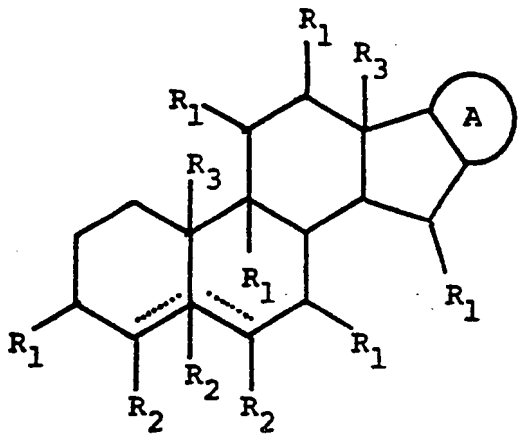
$R_1$ represents hydrogen, amino, oxo or $OR_4$;
$R_2$ represents hydrogen, amino or $OR_4$ provided that $R_2$ represents hydrogen when adjacent a double bond and no more than one $R_2$ is other than hydrogen;
$R_3$ represents hydrogen, $(C_1-C_6)$ alkyl or $R_4O(C_1-C_6)$ alkylene;
$R_4$ represents hydrogen, a tetrose, pentose or hexose, or two,

three, four or more linked units, wherein each unit is independently selected from tetroses, pentoses and hexoses; and

X represents O or NH;

and the pharmaceutically acceptable salts of such compounds; with the proviso that no more than two of $R_1$ and $R_2$ are other than hydrogen.

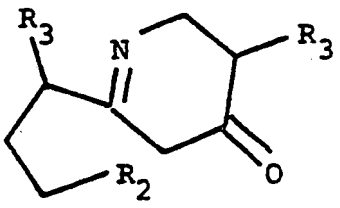
2. Pharmaceutical compositions comprising at least one compound of the formula



(I)

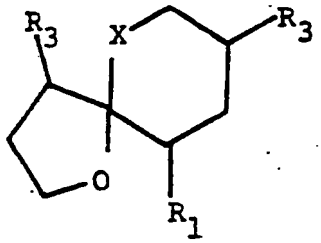
wherein one of the dotted lines represents a double bond, or both represent single bonds;

A represents



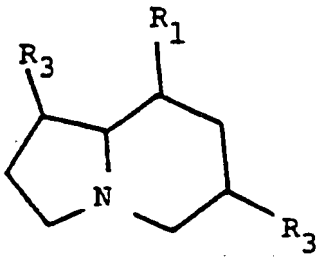
(II)

,



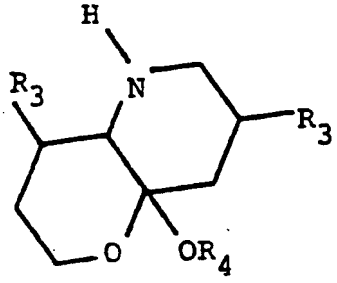
(III)

,



(IV)

or



(V)

;

$R_1$ represents hydrogen, amino, oxo or $OR_4$;

$R_2$ represents hydrogen, amino or $OR_4$ provided that $R_2$ represents hydrogen when adjacent a double bond and no more than one $R_2$ is other than hydrogen;

$R_3$ represents hydrogen, $(C_1-C_6)$ alkyl or $R_4O(C_1-C_6)$ alkylene;

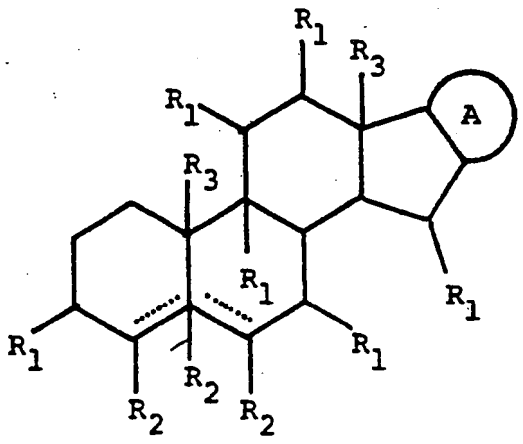
$R_4$ represents hydrogen, a tetrose, pentose or hexose, or two, three, four or more linked units, wherein each unit is independently selected from tetroses, pentoses and hexoses; and

X represents O or NH;

and the pharmaceutically acceptable salts of such compounds; with the proviso that no more than two or $R_1$ and $R_2$ are other than hydrogen, together with a pharmaceutically acceptable carrier.

3. The composition as defined in claim 2 wherein said compound is solasonine, solamargine, solanine or a mixture of two or more thereof or solasodine, solanidine or a mixture there.

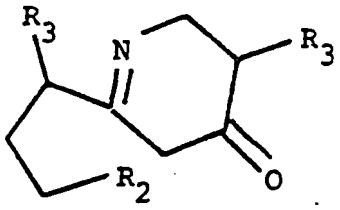
4. The use in the treating or preventing of disease or infection in a subject requiring said treatment of a compound of the formula



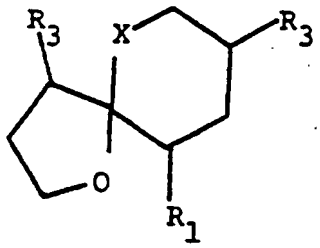

(I)

wherein one of the dotted lines represents a double bond, or both represent single bonds;

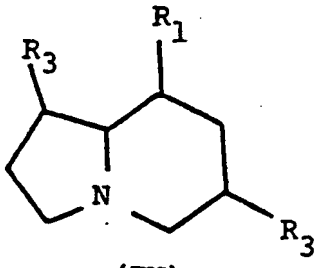
A represents



(II)

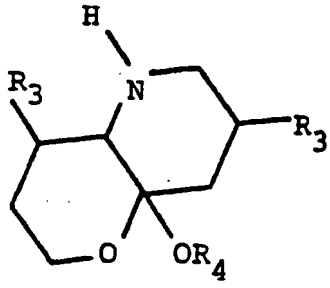


(III)



(IV)

or



(V)

;

$R_1$ represents hydrogen, amino, oxo or $OR_4$;

$R_2$ represents hydrogen, amino or $OR_4$ provided that $R_2$ represents hydrogen, when adjacent a double bond and no more than one $R_2$ is other than hydrogen;

$R_3$ represents hydrogen, $(C_1-C_6)$ alkyl or $R_4O(C_1-C_6)$ alkylene;

$R_4$ represents hydrogen, a tetrose, pentose or hexose, or two, three, four or more linked units, wherein each unit is independently selected from tetroses, pentoses and hexoses; and

X represents O or NH;

with the proviso that no more than two of $R_1$ and $R_2$ are other than hydrogen, or a pharmaceutically acceptable salt of such compound.

5. The use as defined in claim 4 wherein said compound is solasonine, solamargine, solanine or a mixture of two or more thereof or solasodine, solanidine or a mixture thereof.

6. The use as defined in claim 4 or 5 wherein said disease or infection is cancer, tinea, warts, acne, psoriasis, haemarrohoids, skin inflammation or other skin disorder.

7. A process for extracting glycoalkaloids from plants of the Solanum species comprising grinding parts of said plants, subjecting the ground plant matter to the action of at least one dilute acid and making the acid exteact alkaline to precipitate said glycoalkaloids.

8. The process as defined in claim 7 wherein said acid is acetic acid or formic acid.

9. The process as defined in claim 7 or claim 8 wherein said plants are Solanum sodomeum.

10. The process as defined in any one of claims 6 to 9 comprising the steps of:-

(i) grinding said plant material coarsely;

(ii) mixing said ground plant material with dilute acid to form a first acid extract;

(iii) separating the solid residue from step (ii) from said first acid extract;

(iv) mixing said solid residue with further dilute acid to form a second acid extract;

(v) separating the solid residue from step (iv) from said second acid extract;

(v) combining said first and second acid extracts; and

(vii) adding alkali to said combined acid extracts to precipitate said glycoalkaloids.

11. The process as defined in claim 10 wherein said dilute is 2% acetic acid or 2% formic acid.

12. The process as defined in any one of claims 10 or 11 to 10 wherein either or both of steps (ii) and (iv) is/are carried out at ambient temperature for between 2 and 4 hours.

13. The process as defined in any one of claims 10 to 12 wherein said alkali is ammonia.

14. The process as defined in any one of claims 10 to 13 further comprising the step of heating the combined extracts which have been made alkaline in step (vii) to 80°C.

15. The process as defined in any one of claims 10 to 14 wherein said glycoalkaloids so obtained are purified by recrystallisation.

16. The process as defined in any one of claims 7 to 9 comprising the steps of:-

(a) grinding said plant material in the presence of a dilute acid;
(b) diluting the mixture formed thereby with further dilute dilute acid;
(c) agitating said diluted mixture to form an acid extract;
(d) separating the solid residue from step (c) from said acid extract;
(e) warming the said acid extract; and
(f) increasing the pH of said warmed acid extract to 9 to 10 to precipitate said glycoalkaloids.

17. The process as defined in claim 16 comprising the further steps of:-

(g) dissolving said glycoalkaloids in dilute acid;
(h) separating any material which does not dissolve;
(i) warming the resultant solution;
(j) increasing the pH of said warmed solution to 9 to 10 to precipitate said glycoalkaloids;
(k) holding the suspension formed thereby at the temperture to which it is warmed for a short period;
(l) boiling said suspension; and
(m) separating the precipitated glycoalkaloids.

18. The process as defined in claim 17 wherein the glycoalkaloids so obtained are purified by recrystallisation.

19. The process as defined in any one of claims 16 to 18 wherein said dilute acid in any one or more of steps (a), (b) and (g) is 3% acetic acid.

20. The process as defined in any one of claims 16 to 19 wherein step (c) is carried out at ambient temperature for between 18 and 24 hours.

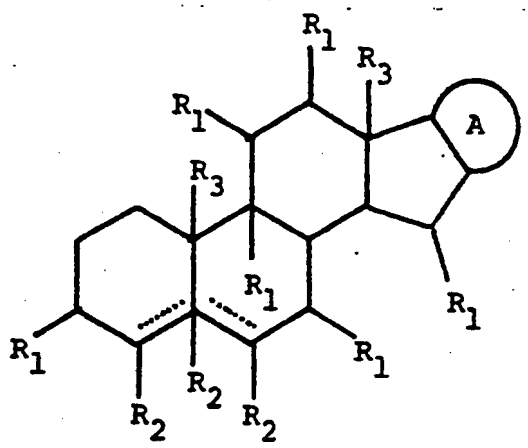
21. The process as defined in any one of claims 16 to 20 wherein, in any one or more of steps (e), (i) and (k), the temperature is 50°C.

22. The process as defined in any one of claims 16 to 21 wherein, in step (f) and/or (j), the pH is increased by addition of ammonia.

23. The process as defined in any one of claims 17 to 21 wherein said short period is 5 minutes.

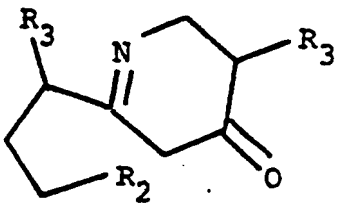
CLAIMS (Austria only);

1. A process for the production of a steriod alkaloid compound or derivative compound thereof effective in the treatment or prevention of disease, which compound or derivative thereof possesses the formula:

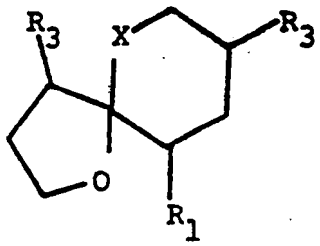


(I)

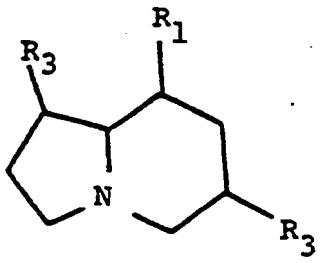
wherein one of the dotted lines represents a double bond, or both represent single bonds;
A represents



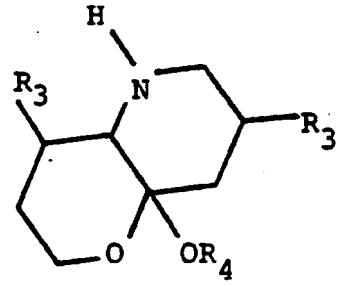
(II)



(III)



or



(IV) (V)

$R_1$ represents hydrogen, amino, oxo or $OR_4$;
$R_2$ represents hydrogen, amino or $OR_4$ provided that $R_2$ represents hydrogen when adjacent a double bond and no more than one $R_2$ is other than hydrogen;
$R_3$ represents hydrogen, $(C_1-C_6)$ alkyl or $R_4O(C_1-C_6)$ alkylene;
$R_4$ represents hydrogen, a tetrose, pentose or hexose, or two,

three, four or more linked units, wherein each unit is independently selected from tetroses, pentoses and hexoses; and

X represents O or NH;

which process comprises grinding parts of plants of the Solanum species, subjecting the ground plant matter to the action of at least one dilute acid, making the acid extract alkaline to precipitate its glycoalkaloid content, isolating the glycoalkaloids as such, after conversion to a pharmaceutically acceptable salt or salts thereof, or after conversion by a steriod alkaloid interconversion method to one or more other said steroid alkaloids which is/are isolated as such or as a pharmaceutically acceptable acid addition salt thereof.

2. The process as defined in claim 1 wherein said acid is acetic acid or formic acid.

3. The process as defined in claim 1 or claim 2 wherein said plants are Solanum sodomeum.

4. The process as defined in any one of claims 1 to 3 comprising the steps of :-

(i) grinding said plant material coarsely;

(ii) mixing said ground plant material with dilute acid to form a first acid extract;

(iii) separating the solid residue from step (ii) from said first acid extract;

(iv) mixing said solid residue with further dilute acid to form a second acid extract;

(v) separating the solid residue from step (iv) from said second acid extract;

(vi) combining said first and second acid extracts; and

(vii) adding alkali to said combined acid extracts to precipitate said glycoalkaloids.

5. The process as defined in claim 4 wherein said dilute acid is 2% acetic acid or 2% formic acid.

6. The process as defined in claim 4 or 5, wherein either or both of step (ii) and step (iv) is carried out at ambient temperature for between 2 and 4 hours.

7. The process as defined in any one of claims 4 to 6 wherein said alkali is ammonia.

8. The process as defined in any one of claims 4 to 7 further comprising the step of heating the combined extracts which have been made alkaline in step (vii) to 80°C.

9. The process as defined in any one of claims 4 to 8 wherein said glycoalkaloids so obtained are purified by recrystallisation.

10. The process as defined in any one of claims 1 to 3 comprising the steps of:-

(a) grinding said plant material in the presence of a dilute acid;
(b) diluting the mixture formed thereby with further dilute dilute acid;
(c) agitating said diluted mixture to form an acid extract;
(d) separating the solid residue from step (c) from said acid extract;
(e) warming the said acid extract; and
(f) increasing the pH of said warmed acid extract to 9 to 10 to precipitate said glycoalkaloids.

11. The process as defined in claim 10 comprising the further steps of:-

(g) dissolving said glycoalkaloids in dilute acid;
(h) separating any material which does not dissolve;
(i) warming the resultant solution;
(j) increasing the pH of said warmed solution to 9 to 10 to precipitate said glycoalkaloids;
(k) holding the suspension formed thereby at the temperture to which it is warmed for a short period;
(l) boiling said suspension; and
(m) separating the precipitated glycoalkaloids.

12. The process as defined in claim 11 wherein the glycoalkaloids so obtained are purified by recrystallisation.

13. The process as defined in any one of claims 10 to 12 wherein said dilute acid in any one or more of steps (a), (b) and (g) is 3% acetic acid.

14. The process as defined in any one of claims 10 to 13 wherein step (c) is carried out at ambient temperature for between 18 and 24 hours.

15. The process as defined in any one of claims 10 to 14 wherein, in one or more steps (e), (i) and (k), the temperature is 50°C.

16. The process as defined in any one of claims 10 to 15 wherein, in step (f) and/or (j), the pH is increased by addition of ammonia.

17. The process as defined in any one of claims 10 to 15 wherein said short period is 5 minutes.

18. A method for the production of a pharmaceutical composition effective in the treatment or prevention of disease or infection, which comprises placing in admixture with a pharmaceutically acceptable carrier, a compound produced by the process claimed in any one of the preceding claims.

19. A method as claimed in claim 18, wherein said compound is solasonine, solamargine, solanine or a mixture of two or more thereof, or solasodine, solanidine or a mixture thereof.

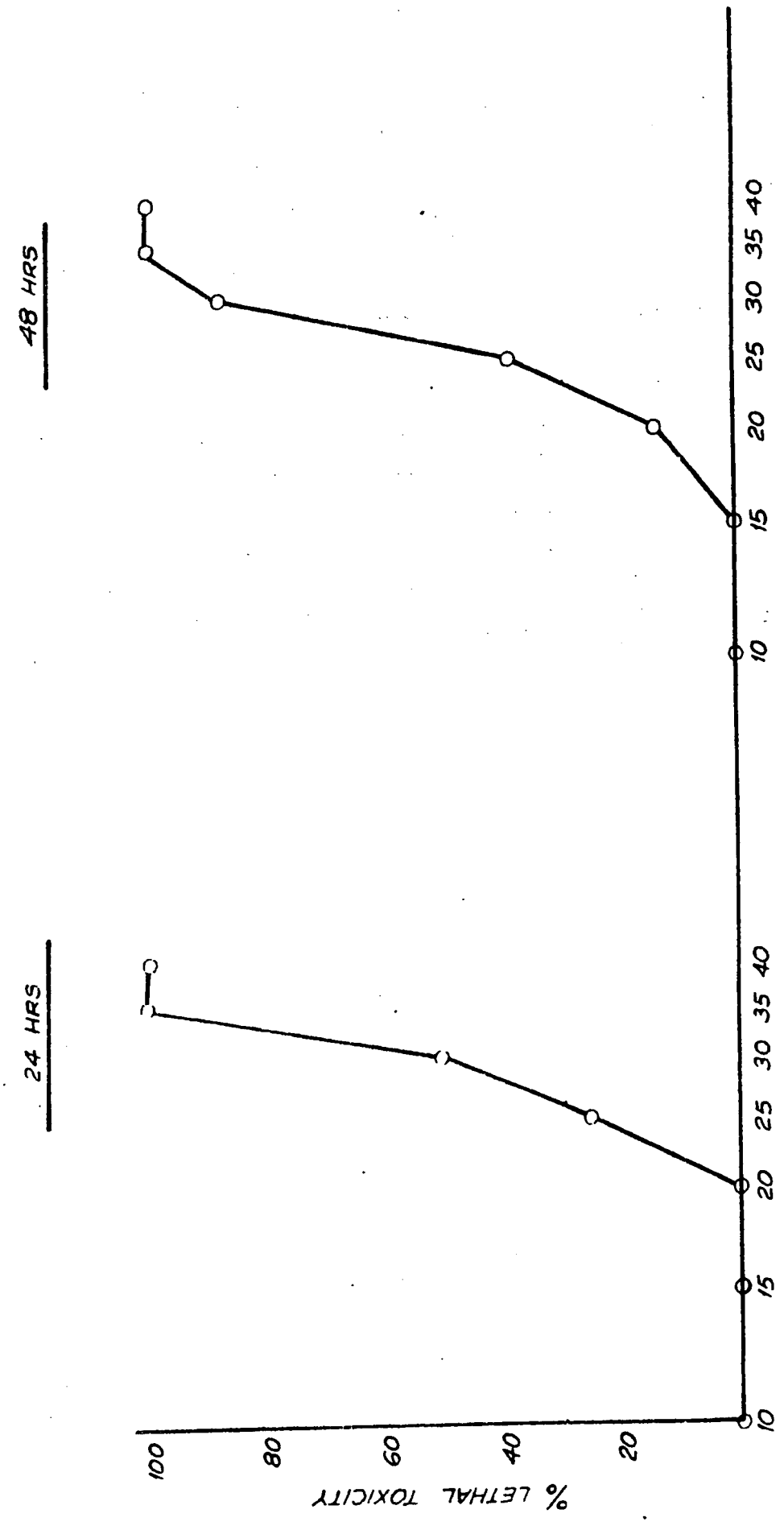

48 HRS
24 HRS
% LETHAL TOXICITY
100
80
60
40
20
10
15
20
25
30
35
40
CONC. BEC 001 mg/kg

FIG. 1

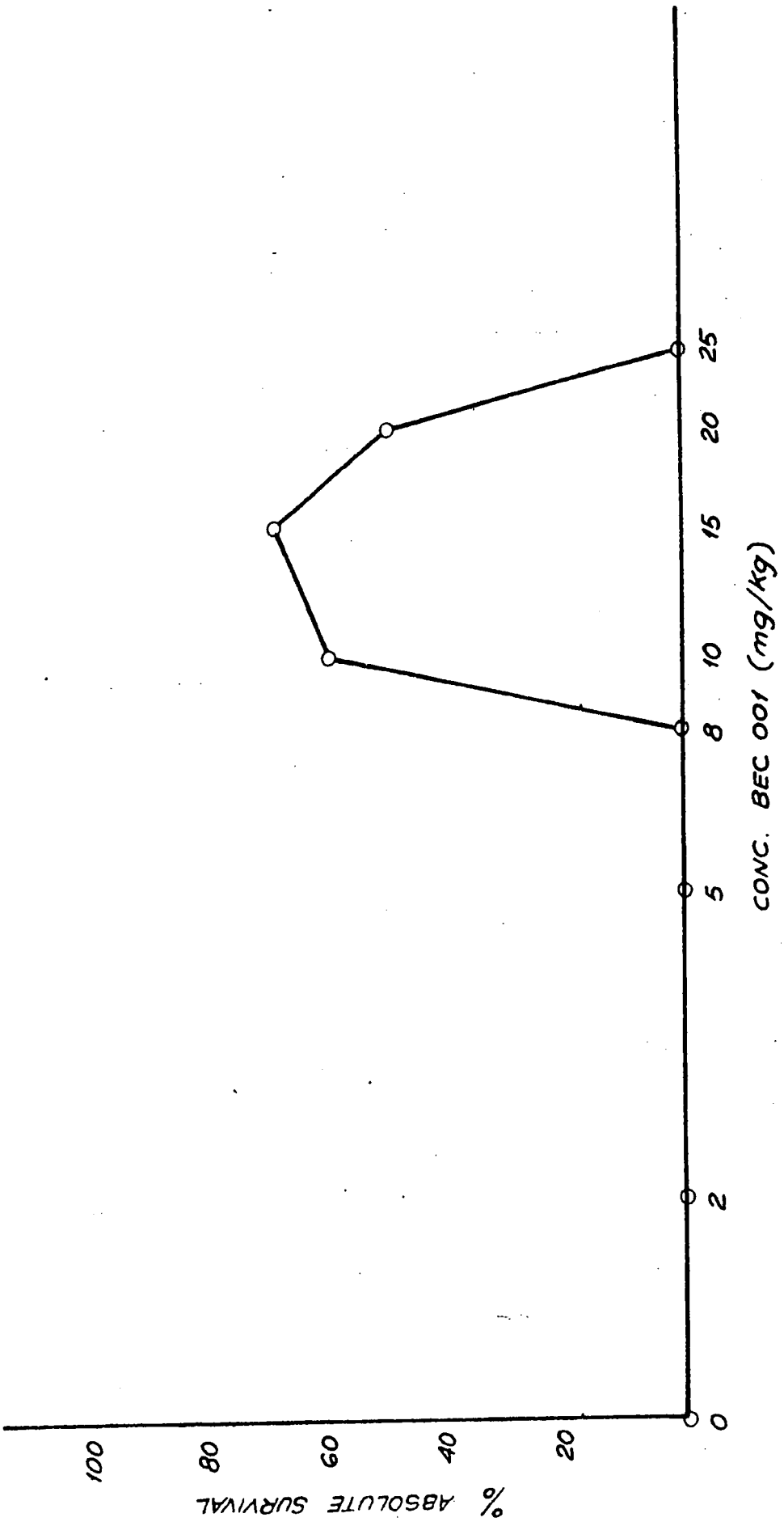

% ABSOLUTE SURVIVAL
100
80
60
40
20
0
2
5
8
10
15
20
25
CONC. BEC 001 (mg/kg)

FIG. 2

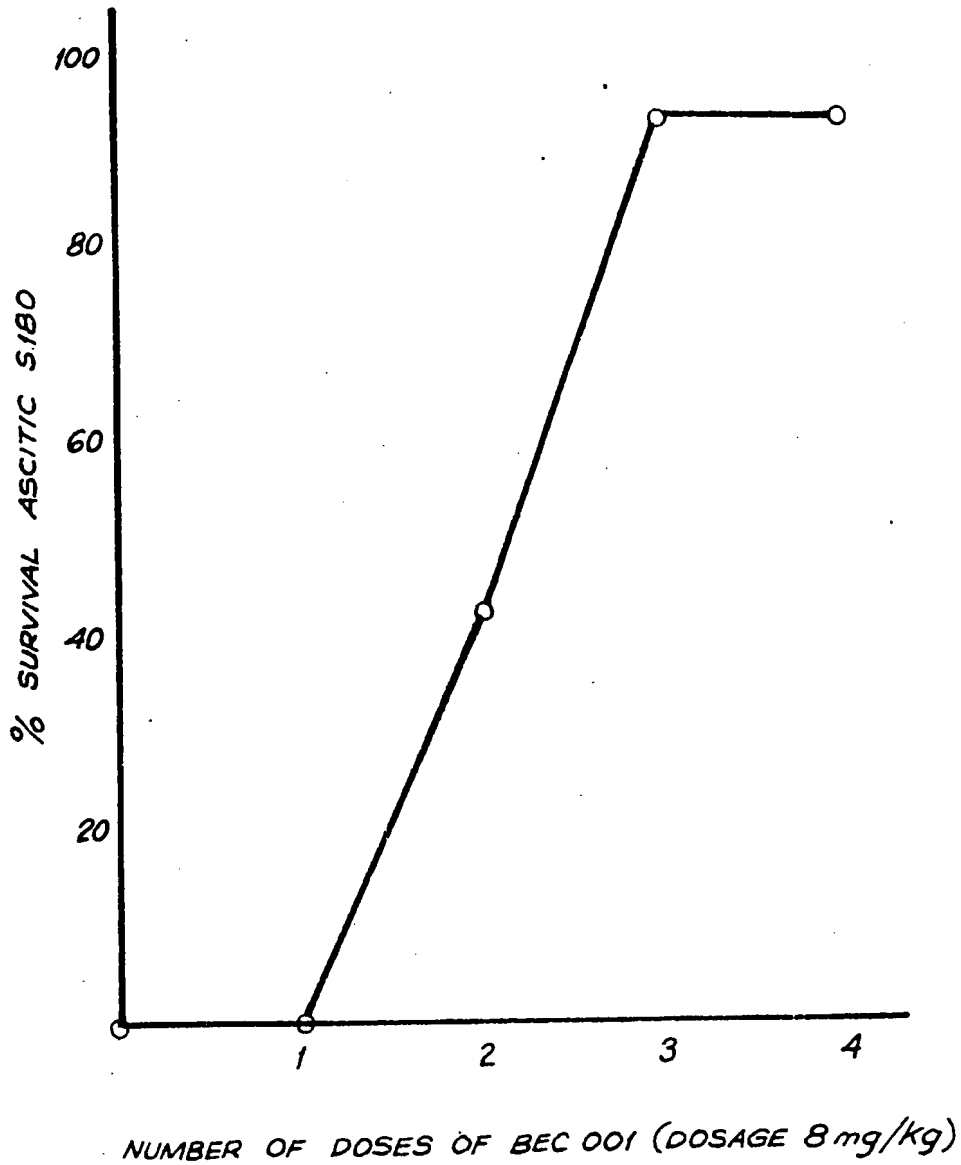

100
80
60
40
20
% SURVIVAL ASCITIC S.180
1
2
3
4
NUMBER OF DOSES OF BEC 001 (DOSAGE 8 mg/kg)

FIG. 3

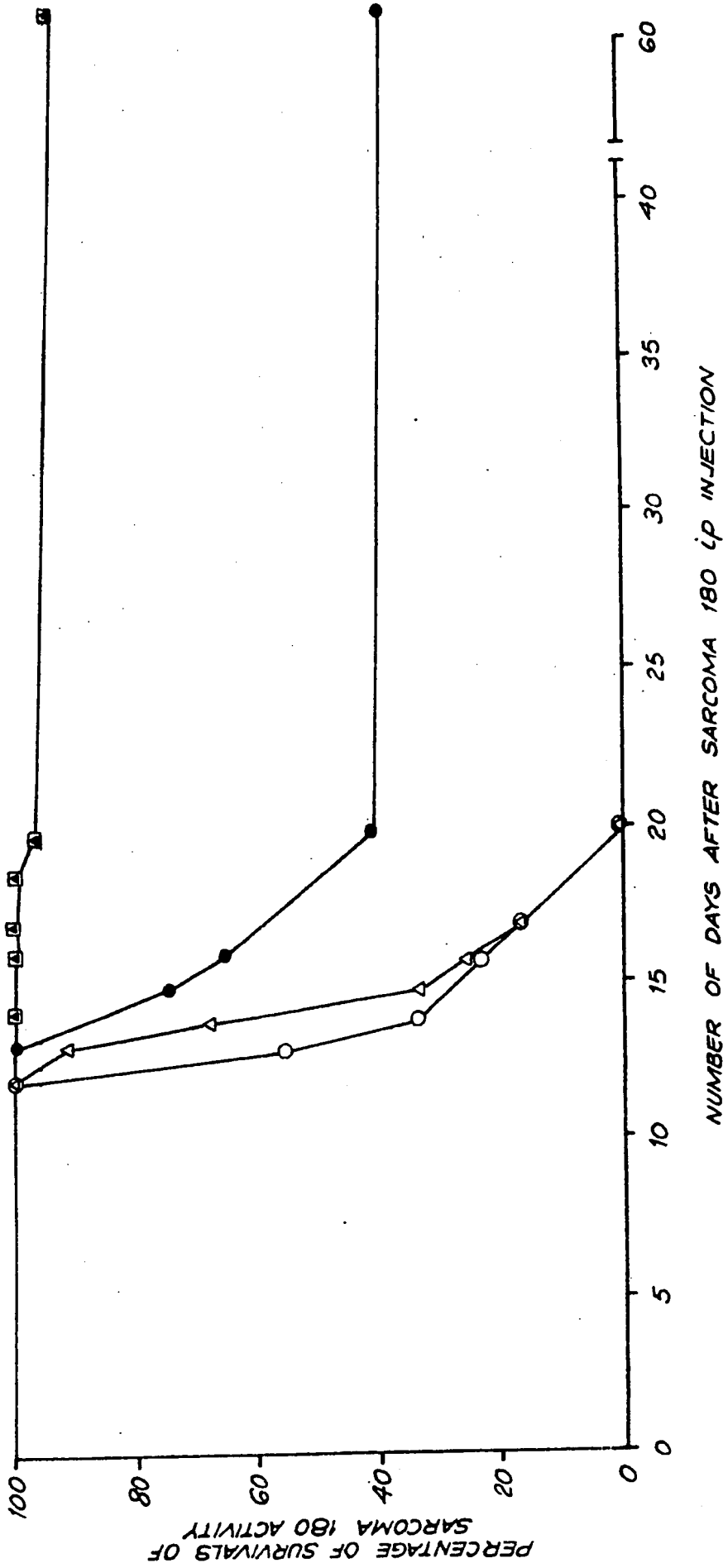

PERCENTAGE OF SURVIVALS OF SARCOMA 180 ACTIVITY
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
60
NUMBER OF DAYS AFTER SARCOMA 180 IP INJECTION

FIG. 4

European Patent Office

# EUROPEAN SEARCH REPORT

0020029
Application number
EP 80 30 1443

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim |
|---|---|---|
| X | CHEMICAL ABSTRACTS, vol. 68, no. 5, 29-01-1968, page 1975, abstract 20709v. Columbus, Ohio, USA C. BODEA et al. "Antimitotoic activity of solasodine and some derivatives". & Rev. Roum. Biochim. 4(3), 169-174, (1967) * Abstract * -- | 1-6 |
| X | FR - M - 2895 (LABORATOIRES JOUVEINAL) * Summary * -- | 1,2,4, 6 |
| X | FR - M - 5211 (GEORGES SCHUSTER) * Summary * -- | 1,2,4, 6 |
| X | FR - M - 2360 (LABORATOIRES JOUVEINAL) * Summary * -- | 1,2,4, 6 |
| X | FR - M - 2359 (LABORATOIRES JOUVEINAL) * Summary * -- | 1,2,4, 6 |
| X | FR - M - 2367 (LABORATOIRES JOUVEINAL) * Summary * -- | 1,2,4, 6 |

./.

CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)

C 07 J 71/00
43/00
A 61 K 31/50
31/70
35/78

TECHNICAL FIELDS SEARCHED (Int.Cl. 3)

C 07 J 71/00
A 61 K 31/58
A 61 K 31/70

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons

&: member of the same patent family, corresponding document

X The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| The Hague | 24-07-1980 | HENRY |

EPO Form 1503.1 06.78

| EINSCHLÄGIGE DOKUMENTE | | | KLASSIFIKATION DER ANMELDUNG (Int.Cl. ³) |
|---|---|---|---|
| Kategorie | Kennzeichnung des Dokuments mit Angabe, soweit erforderlich, der maßgeblichen Teile | betrifft Anspruch | |
| X | ARZNEIMITTELFORSCHUNG, vol. 26, no. 1, January 1976, Aulendorf, DE J. KULCSAR-GERGELY: "The effect of solasodine on the body temperature", pages 55-57. * Whole article * -- | 1-6 | |
| X | CHEMICAL ABSTRACTS, vol. 75, no.7, 16-08-1971, page 222, abstract 47319z. Columbus, Ohio, USA K. NISHIE et al. "Pharmacology of solanine". & Toxicol. Appl. Pharmacol. 1971, 19(1), 81-92. * Abstract * -- | 1-6 | RECHERCHIERTE SACHGEBIETE (Int. Cl. ³) |
| X | CHEMICAL ABSTRACTS, vol. 59, no. 12, 9-12-1963, page 14053, abstract 14053d-h. Columbus, Ohio, USA KLAUS SCHREIBER et al. "Solanum alkaloids XXV. Identification of tomatine as a principal alkaloid of the wild potato Solanum demissum". & Z. Natur forsch, 18b, 471-5, (1963). * Abstract * -- | 7,10, 11, 13-23 | |
| X | CHEMICAL ABSTRACTS, vol. 5, no. 20, 20-10-1911, pages 3406-3407. Columbus, Ohio, USA GUISEPPE ODDO et al. "Solanine-extracted from Solanum sodomaeum". | 7.9 ./. | |

| EINSCHLÄGIGE DOKUMENTE | | | KLASSIFIKATION DER ANMELDUNG (Int.Cl. ³) |
|---|---|---|---|
| Kategorie | Kennzeichnung des Dokuments mit Angabe, soweit erforderlich, der maßgeblichen Teile | betrifft Anspruch | |
| | & Gazz. Chim. Ital. 41I, 490-534 and 35I 28, 36I 310, 36II 522 * Abstract * | | RECHERCHIERTE SACHGEBIETE (Int. Cl. ³) |